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(54) Title: MODIFICATION OF SUCROSE SYNTHASE GENE EXPRESSION IN PLANT TISSUE AND USES THEREFOR

(57) Abstract: Methods and means are provided to modulate fibre quality in fibre-producing plants, such as cotton by modulating sucrose synthase activity and/or expression in such plants. The methods and means may also be used to obtain plants with seedless fruits or male-sterile plants.

Modification of sucrose synthase gene expression in plant tissue and uses therefor

Technical Field

The present invention relates to modifying targeted gene expression in plants to obtain a desired effect in the plant.

Background Art

Sucrose synthase (SuSy) is a key enzyme in the breakdown of sucrose in all plant sink tissue, including grain and fruit and has been extensively studied in many plants. Only relatively recently, however, has this protein and gene been characterised from cotton. The full length (2625 bp) of cotton sucrose synthase (SuSy) was isolated by Perez-Grau, L and Delmer, D in UC, Davis (accession number U73588) in May 1996. A 2030 bp fragment of the same cDNA with 595 bp missing at the 5' end was isolated by the same group in 1994 and was given to Prem Chourey in USDA/ARS for collaborative research. However, no evidence was available at that time regarding the role of this SuSy gene in cotton fibre/seed development, although it had been speculated (Amor *et al.*, 1995) that part of the fibre localised SuSy could associate with cellulose synthase playing a role to channel carbon to this enzyme.

Evidence has been obtained that the expression of the SuSy gene could be important not only for cellulose synthesis but also for fibre cell initiation (thus may control fuzz) and a model on how sucrose is partitioned and competed for between fibre, seed coat and embryos of the cotton seed was proposed (Ruan *et al* 1997 *Plant Physiol. 115*, 375-385; Ruan and Chourey 1998 *Plant Physiol. 118*, 399-406). More recently, the present inventors obtained further evidence supporting the hypothesis that SuSy plays a key role in mobilising sucrose into initiating fibre cells (Ruan *et al.*, 2000 *Aust. J. Plant Physiol.* 27, 795-800).

The art is thus deficient in providing methods and means for altering the fibre development and properties in plants, particularly cotton, through alteration of

sucrose synthase levels in cells of the plants. The present inventors have now shown that it is possible to modify one or more fibre characteristics and/or fibre content by modifying SuSy gene expression, particularly by modifying SuSy expression in transgenic cotton.

These and other problems are solved as described hereinafter in the different embodiments and claims.

Throughout this specification, unless the context requires otherwise the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps but not the exclusion of any other element, integer or step or group of elements, integers or steps.

Summary of the invention.

The invention provides a method for altering fibre development or properties of a fibre producing plant, preferably a cotton plant, such as, for example, a cotton plant of the variety fribremax and particularly the Fibermax™ variety, said method comprising the steps of providing cells of said plants with a chimeric gene comprising the following operably linked DNA fragments: a plant operable promoter, preferably a subterranean clover stunt virus promoter; a coding region which when transcribed yields an RNA said RNA being capable of reducing the expression of an endogenous sucrose synthase gene, preferably an endogenous sucrose synthase gene expressed in fibre cells, preferably fibre initial cells, or capable of being translated into an active sucrose synthase protein; and transcription termination and polyadenylation signals that function in the plant cells.

In the embodiments where the RNA is capable of being translated into an active sucrose synthase protein preferred coding regions comprise a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2;

(b) a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 1; (c) a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of (a) or (b); and (d) a nucleotide sequence hybridizing under stringent conditions with the complement of (a) or (b); and (e) a fragment of any one of (a) through (d) encoding an active sucrose synthase.

In the embodiments where the RNA is capable of reducing the expression of an endogenous sucrose synthase gene, preferred coding regions comprise a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence comprising at least about 19 or 25 contiguous nucleotides having at least 70% sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or the complement thereof; and (b) a nucleotide sequence comprising at least about 19 or 25 contiguous nucleotides having at least 70% sequence identity from a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or the complement thereof. More particularly, the coding region comprises the nucleotide sequence of SEQ ID NO: 1 from the nucleotide at about position 2208 to the nucleotide at about position 2598, or the complement thereof. The coding region may comprise simultaneously both sense and antisense nucleotide sequences capable of forming a double stranded RNA molecule.

It is another objective of the invention to provide a method for improving fibre yield in a fibre producing plant, comprising the steps of providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments: (a) a plant operable promoter; (b) a DNA region capable of being translated into an active sucrose synthase protein; and (c) transcription termination and polyadenylation signals that function in said plant cells.

The invention also provides a method for improving fibre quality in a fibre producing plant, comprising the steps of providing cells of said plant with a

chimeric gene comprising the following operably linked DNA fragments: (a) a plant operable promoter; (b) a DNA region capable of being translated into an active sucrose synthase protein; and (c) transcription termination and polyadenylation signals that function in said plant cells.

In yet another embodiment of the invention a method is provided for increasing seed size in a fibre producing plant, comprising the steps of providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments: (a) a seed-specific promoter; (b) a DNA region capable of being translated into an active sucrose synthase protein; and (c) transcription termination and polyadenylation signals that function in said plant cells.

The invention also relates to fibre producing plants, particularly cotton plants, comprising in their genome a chimeric DNA comprising the following operably linked DNA fragments: (a) a plant operable promoter; (b) a coding region which when transcribed yields an RNA, said RNA being capable of reducing the expression of an endogenous sucrose synthase gene, preferably an endogenous sucrose synthase gene expressed in fibre cells, preferably fibre initial cells, or alternatively, which is capable of being translated into an active sucrose synthase protein; and (c) transcription termination and polyadenylation signals that function in said plant cells.

Yet another objective of the invention is to provide a fibre producing plant comprising a chimeric DNA according to the invention wherein said DNA is transcribed to yield RNA that is capable of increasing the expression of an endogenous sucrose synthase gene, preferably an endogenous sucrose synthase gene expressed in fibre cells, preferably fibre initial cells and said fibre cells have an increased sucrose synthase activity compared to fibre cells of plant cells which do not comprise said chimeric DNA.

Yet another objective of the invention is to provide a fibre producing plant comprising a chimeric DNA according to the invention, wherein said chimeric DNA is transcribed to yield RNA that is capable of being translated into an active sucrose synthase protein, thereby increasing the level of sucrose synthase in the plant relative to a plant that does not comprise said chimeric DNA. Preferably, the active sucrose synthase is expressed in fibre cells, more preferably fibre initial cells, wherein said fibre cells preferably have higher sucrose synthase activity compared to the fibre cells of a plant which does not comprise the chimeric DNA.

In yet another embodiment of the invention fibre producing plants are provided comprising a chimeric DNA according to the invention, wherein said coding region comprises a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2; (b) a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 1; (c) a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence of (a) or (b); (d) a nucleotide sequence hybridizing under stringent conditions with the nucleotide sequence of (a) or (b); and (e) a fragment of any one of (a) through (d) encoding an active sucrose synthase.

The invention also provides a fibre producing plant comprising the chimeric DNA of the invention wherein said chimeric DNA encodes an RNA capable of reducing an endogenous sucrose synthase gene expression, such that said fibre cells have a reduced sucrose synthase activity compared to fibre cells of plant cells that do not comprise said chimeric DNA, particularly wherein the coding region comprises a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence comprising at least about 19 or 25 contiguous nucleotides having at least 70% sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or the complement thereof; and (b) a nucleotide sequence

comprising at least about 19 or 25 contiguous nucleotides having at least 70% sequence identity from a nucleotide sequence encoding a polypeptide comprising the nucleotide sequence of SEQ ID NO: 1 or the complement thereof; and (c) the nucleotide sequence of SEQ ID NO: 1 from the nucleotide at about position 2208 to the nucleotide at about position 2598 or the complement thereof.

Also provided are seeds of a fibre producing plant, preferably the seed of a cotton plant comprising the chimeric DNA or genes according the invention, as well as fibres with altered development or properties, isolated from such plants.

The invention also relates to the use of a sucrose synthase encoding nucleotide sequence for altering fibre yield or fibre quality or fibre properties.

Brief Description of Drawings

Figure 1 is a photographic representation showing that down regulation of SuSy in the fibre causes a decrease in fibre elongation in transgenic plants. Transverse sections of cotton bolls and seeds of wild type (WT) and transgenic cotton carrying an antisense SuSy gene construct (SuSy/A) are shown. Three developmental stages are shown: 0, 2 and 6 days after anthesis. Data indicate reduced fibre in transgenic lines, particularly in samples 2 days post anthesis (DAA) or older.

Figure 2 is a photographic representation of an immunohistochemical stain showing localisation of SuSy protein in wild type (WT) cotton (panel A), and the transgenic lines 291-147 (panel B) and 295-82 (panel C). Black coloration in developing fibre cells on the surface of the ovule, and in the ovule epidermis, of the wild type (WT) plants indicates SuSy protein. Transgenic lines show reduced amount of SuSy protein (reduced black coloration). No SuSy protein was detectable in line 295-82. This reduced SuSy protein correlates with lack of fibre development in the transgenic lines.

Figure 3 is a photographic representation of cotton bolls (left) and seed (right) from wild type (WT) cotton and the transgenic line 294-147 carrying an antisense SuSy gene construct, which has reduced SuSy protein in the embryo (upper boll and seeds). Seeds from the transgenic line are fibreless, but there is also a great reduction in seed size in seeds labeled III. Some seeds did not form at all.

Figure 4 is a photographic representation of wild type cotton plants (left of the figure) and two transformed cotton lines (plants at the middle and right of the figure). Data show that there was no detectable phentotypic effect of the transgene with respect to vegetative growth of plants.

Figure 5 is a schematic representation of an antisense gene construct (top) and a cosuppression gene construct (below) used to suppress SuSy gene expression *in planta*. Gene construct components are as follows: nptII, neomycin phosphotransferase marker gene for selection of transformants; S1 and S7, subclover stunt virus promoters 1 and 7, respectively (see Australian Patent No 689311); S3 and S5, subclover stunt virus gene terminator sequences 3 and 5, respectively (see Australian Patent No 689311); SuSy, a 390 bp nucleotide sequence of a sucrose synthase-encoding cDNA (Genbank Accession number U73588) from CTGGGAT to TGACTT (ie. 211 bp coding region upstream of the stop codon plus 179 bp 3'-untrnaslated (UTR) region; nucleotide position 2208 to nucleotide position 2598 of SEQ ID NO: 1). Arrows indicate the orientation of fragments relative to their native orientation in the genes from which they were derived.

Figure 6 is a copy of a photographic representation of a Southern blot hybridization, showing SuSy gene copy in wild type cotton plants and transgenic cotton lines. DNAs were isolated from T0 generation plants (panel A), and a segregating population of T1 generation plants from the primary

transformed lines designated 14 and 4 (panel B). The arrows indicate lines 14, 8, and 4 in the T0 generation having a fibre-less or reduced fibre phenotype (panel A), and lines 14-1, 14-2, 14-3, 4-1, and 4-3 in the segregating population of T1 plants having a fibre-less or reduced fibre phenotype (panel B). The numbers indicate the transgenic cotton line from which the individual plants were derived.

Figure 7 is a photographic representation of a scanning electron microscopy (SEM) profile of the ovule epidermis of wild type cotton (panel A) and line 294-147 shown in Fig. 2B (panels B and C). Many fibre cells are collapsed and/or shrunken in the transgenic line, compared to the wild type plants. Whilst some fibre cells appeared in the transgenic lines, these were much smaller in size and fewer in number than in the wild type ovule (compare panels A and B). The shrunken and/or collapsed phenotype of the trasngenic line was clearly demonstrated under high magnification (panel C).

Figures 8, 9 and 10 shows the correlation between changes of SuSy activity (Fig. 8) and reduction of fibre length (Fig. 9) in 3 day old seeds from 10 segregating individuals of three T1 generation lines (lines 147, 43 and 101), together with line 82 and wild type (WT). Figure 10 represents the regression analysis between SuSy activity and fibre length.

Figure 8 is a graphical representation showing modified SuSy activity (abscissa) in transgenic lines compared to wild type plants. Plant lines are indicated on the x-axis. Error bars represent the SEM for assays of 3-day old seeds from 10 segregating individuals of the T1 generation lines.

Figure 9 is a graphical representation showing modified fibre length (abscissa) in transgenic lines compared to wild type plants. Plant lines are indicated on the x-axis. Error bars represent the SEM for assays of 3-day old seeds from 10 segregating individuals of the T1 generation lines.

Figure 10 is a graphical representation of a regression analysis between SuSy activity data rpesented in Figure 8 (x-axis) and fibre length data presented in Figure 9 (abscissa). Data show a strong correlation (R^2=0.98) between SuSy activity and fibre length over the range shown.

Figure 11 is a photographic representation showing fuzz fibre length in seeds of wild type cotton (panel A) and the transgenic line 147#2 (panel B), and showing reduced fuzz in the transgenic line. Data demonstrate that a reduction in SuSy gene expression reduces fuzz. Seed were delinted by hand. The oval represents the chalazal end of the mature seeds. Seed from the transgenic line are more brown in appearance than wild type seeds, because the underlying brown seed coat is covered by shorter fuzz fiber than in wild type seed.

Detailed Description of the Invention

In a first aspect, the present invention provides a plant having altered expression of an iso-form of sucrose synthase (SuSy) resulting in an altered fibre, fruit or seed production ability.

Preferably, the plant is a transgenic plant having an under- or overexpression of an iso-form of SuSy involved in fruit/seed production. In a preferred form, the plant retains expression of SuSy in developing vegetative tissue such that growth and development of the plant is not adversely affected.

When the expression of an isoform of SuSy that is involved in fruit/seed production is reduced in the plant, preferably in the seed, more preferably in the fibre producing cells, even more preferably in fibre initial cells, the plant has a reduced fibre and/or seed production. When the expression of an isoform of SuSy that is involved in fruit/seed production is enhanced (i.e. SuSy is over-expressed) in the plant, preferably in the seed, more preferably in the fibre

cells, even more preferably in fibre initial cells, the plant has enhanced fibre and/or seed production.

The ability to cause underexpression of an iso-form of SuSy involved in fruit/seed production can be used to develop plants which produce few or no seeds but are still able to grow and produce fruit normally. The ability to cause overexpression of an iso-form of SuSy involved in fruit/seed production in a tissue specific manner can be used to develop plants which produce greater amounts of fibre or longer fibre or having altered fibre structure but are still able to grow and develop normally. The plant can be any plant in which the expression of an iso-form of SuSy is involved in fruit/seed production. The present invention is applicable for modifying a wide range of horticultural crops such as grape, peach, pear, and apple. More preferably, the plant is a cotton plant.

In a preferred embodiment, plants, particularly cotton plants, are provided comprising a chimeric gene, preferably stably integrated in their genome, the chimeric gene comprising the following operably linked DNA fragments:

a) a plant operable promoter
b) a coding region which when transcribed yields an RNA which is either
    i) capable of reducing the expression of an endogenous sucrose synthase gene, preferably an endogenous sucrose synthase gene expressed in fibre initial cells; or
    ii) capable of being translated into an active sucrose synthase protein; and
c) transcription termination and polyadenylation signals that function in plant cells.

A particularly preferred embodiment of the coding region as defined sub ii) is a coding region comprising a nucleotide sequence which encodes a protein comprising the amino acid of SEQ ID NO: 2, particularly the nucleotide

sequence of SEQ ID NO: 1, or a fragment of such a nucleotide sequence capable of being translated into a functional sucrose synthase. The coding region encoding the sucrose synthase activity may be derived from other plant species or other species (as indicated elsewhere in this application), such as, for example, from potato (cDNA sequence is available from Genbank library, accession number M18745).

A particularly preferred embodiment of the coding region as defined sub i) is a coding region comprising the complement of a nucleotide sequence ("antisense") encoding a polypeptide encoded by the nucleotide sequence of SEQ ID NO: 1 from the nucleotide at about position 2208 to the nucleotide at about position 2598, particularly the coding region comprises the nucleotide sequence of SEQ ID NO: 1 from the nucleotide at position 2208 to the nucleotide at position 2598.

As used herein, the term "coding region" shall be taken to mean a DNA sequence which is capable of being transcribed into a biologically active RNA, irrespective of whether or not said DNA sequence encodes an amino acid sequence. In the present context, a biologically active RNA includes RNA which is capable of inducing a biological effect in the target cell, such as antisense or sense RNA capable of triggering post transcriptional gene silencing, or a ribozyme etc.

Biologically active RNA also includes RNA which is capable of being translated into a polypeptide or protein.

Having read these preferred embodiments, the person skilled in the art will immediately realize that functional equivalents of the mentioned coding regions may be used to similar effect.

Functional equivalents of a coding region capable of being transcribed into RNA which is capable of reducing the expression of an endogenous sucrose synthase gene, include e.g. shorter antisense fragments compared to the above-mentioned nucleotide sequences.

The length of the antisense nucleotide sequence may vary from about 10 nucleotides (nt) or 19 nt, up to a length equaling the length (in nucleotides) of the target nucleic acid (i.e. the full-length sucrose synthase gene). Preferably, the total length of the antisense nucleotide sequence is at least 10 nt, preferably 15 nt or 19 nt, more preferably at least about 50 nt, more preferably at least about 100 nt, more preferably at least about 150 nt, more preferably at least about 200 nt, and even more preferably at least about 500 nt. It is expected that there is no upper limit to the total length of the antisense nucleotide sequence, other than the total length of the target nucleic acid. However for practical reasons (such as e.g. stability of the chimeric genes) it is expected that the length of the antisense nucleotide sequence should not exceed 5000 nt, and preferably should not exceed 2500 nt. Conveniently, the length of an antisense nucleic acid is limited to about 1000 nt.

It will be appreciated that the longer the total length of the antisense nucleotide sequence is, the less stringent the requirements for sequence identity between the total antisense nucleotide sequence and the complement of the corresponding sequence in the target sucrose synthase gene become.

Preferably, the total antisense nucleotide sequence should have a sequence identity of at least about 75% with the complement corresponding target sequence, particularly at least about 80 %, more particularly at least about 85%, quite particularly about 90%, especially about 95%, more especially about 100%, quite especially be identical to complement of the corresponding part of the target nucleic acid. However, it is preferred that the antisense nucleotide sequence always includes a sequence of about 10 consecutive nucleotides,

particularly about 19 or 20 nt, more particularly about 50 nt, especially about 100 nt, quite especially about 150 nt with 100% sequence identity to the complement of the corresponding part of the target nucleic acid. Preferably, for calculating the sequence identity and designing the corresponding antisense sequence, the number of gaps should be minimized, particularly for the shorter antisense sequences.

Particularly preferred are antisense nucleotide sequences having a sequence identity of at least about 75%, preferably at least about 80%; particularly at least about 85%; quite particularly about 90%, especially about 95% with the complement of the corresponding part of the nucleotide sequence of SEQ ID NO: 1.

For the purpose of this invention, the "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e. a position in an alignment where a residue is present in one sequence but not in the other is regarded as a position with non-identical residues. The alignment of the two sequences is performed by the Needleman and Wunsch algorithm (Needleman and Wunsch 1970) Computer-assisted sequence alignment, can be conveniently performed using standard software program such as GAP which is part of the Wisconsin Package Version 10.1 (Genetics Computer Group, Madison, Wisconsin, USA) using the default scoring matrix with a gap creation penalty of 50 and a gap extension penalty of 3.

It goes without saying that sense fragments may also be used for reducing the expression of an endogenous sucrose synthase gene, and the same embodiments of length and sequence homology as herein described for antisense molecules, apply mutatis mutandis to the sense molecules.

Particularly suited for reducing the expression of an endogenous sucrose synthase genes are DNA regions, preferably under the control of a plant-operable promoter, which when transcribed result in so-called double stranded RNA molecules, comprising both sense and antisense sequences which are capable of forming a double stranded RNA molecule as described in WO 99/53050 (incorporated herein in its entirety by reference). In this particular case, a chimeric gene may thus be introduced into a plant cell comprising a plant operable promoter operably linked to a DNA region, whereby that DNA region comprises a part of coding region comprising at least 10 or 19 consecutive nucleotides from the coding region of a nucleic acid encoding a sucrose synthase protein, such as but not limited to, a sucrose synthase protein with the amino acid sequence of SEQ ID NO: 1 (the so-called sense part) as well as a DNA sequence which comprises at least the complementary DNA sequence of at least 10 or 19 nucleotides of the sense part, but which may be completely complementary to the sense part (the so-called antisense part). The chimeric gene may comprise additional regions, such as a transcription termination and polyadenylation region functional in plants. When transcribed an RNA can be produced which may form a double stranded RNA stem between the complementary parts of the sense and antisense region. A spacer region may be present between the sense and antisense nucleotide sequence. The chimeric gene may further comprise an intron sequence, preferably located in the spacer region.

Functional equivalents of coding regions capable of being transcribed into RNA which can be translated into an active sucrose synthase comprise mutant or allelic forms derived from sucrose synthase genes, particularly from sucrose synthase gene with active expression in fibre initials.

Methods to derive mutants e.g. of a sucrose synthase gene, particularly of sucrose synthase genes encoding a protein as represented in SEQ ID NO: 2, quite particularly of sucrose synthase gene comprising the nucleotide sequence

of SEQ ID NO: 1, such a site-specific mutagenesis methods are well known in the art, as well as assays to identify active sucrose synthase enzymes encoded by the mutant sequences.

Allelic variants of the nucleotide sequences encoding sucrose synthase may be identified by hybridization of libraries, under stringent conditions, such as cDNA or genomic libraries of a different varieties or plant lines, particularly cotton varieties and plant lines. Nucleotide sequences which hybridize under stringent conditions to nucleotide sequences encoding the amino acid sequence of SEQ ID NO: 2 or to the nucleotide sequence of SEQ ID NO: 1, or a sufficiently large part thereof (preferably about 25 contiguous nucleotides, particularly at least about 50 contiguous nucleotides, more particularly at least about 100 contiguous nucleotides) and which encode a functional protein with sucrose synthase activity are functional equivalents of the above mentioned preferred coding regions. Such nucleotides may also be identified and isolated using e.g. polymerase chain reaction amplification using an appropriate pair of oligonucleotides having at least about 25 contiguous nucleotides, particularly at least about 50 contiguous nucleotides, more particularly at least about 100 contiguous nucleotides of the nucleotide of SEQ ID NO: 1.

"Stringent hybridization conditions" as used herein mean that hybridization will generally occur if there is at least 95% and preferably at least 97% sequence identity between the probe and the target sequence. Examples of stringent hybridization conditions are overnight incubation in a solution comprising 50% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared carrier DNA such as salmon sperm DNA, followed by washing the hybridization support in 0.1 x SSC at approximately 65 °C. Other hybridization and wash conditions are well known and are exemplified in Sambrook *et al*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly chapter 11.

The following are accession numbers of nucleotide sequences in the Genbank library which are plant sucrose synthase genes, parts thereof or nucleotide sequences having sequence similarity to sucrose synthase genes which could be used according to the methods herein described: BM094593 (Glycine max), BM093753 (Glycine max) BM093158 (Glycine max) BM092695(Glycine max) BM092443(Glycine max) BM092322(Glycine max) BM085310(Glycine max) BM085020 (Glycine max) AY059416 (Zea mays) AF273253(Beta vulgaris) L39940 (Oryza sativa) AJ316590 (Nostoc punctiforme) AJ316589 (Nostoc punctiforme) AJ316596 (Anabaena sp) AJ316595(Anabaena sp) AJ316584 (Anabaena sp) BM005654 (Crocus sativus) BI973032 (Glycine max) BI971794 (Glycine max) AF367450 (Prunus persica) BI945506 (Glycine max) BI944973 (Glycine max) AF420224 (Carica papaya) BI788449 (Glycine max) BI788359 (Glycine max) BI787127 (Glycine max) BI787033(Glycine max) BI787000 (Glycine max) BI786823(Glycine max) BI784933 (Glycine max) BI784627 (Glycine max) BI700214 (Glycine max) BI699934 (Glycine max) BI699923 (Glycine max) BI699585(Glycine max) BI543240 (Sugar beet) BI498340 (Glycine max) BI471463 (Glycine max) BI427241(Glycine max) BI427174 (Glycine max) BI427022 (Glycine max) BI426915 (Glycine max)AF393809 (Apium graveolens) BI321173 (Glycine max) BI320832(Glycine max) BI316894 (Glycine max) BI316826 (Glycine max) BI316405 (Glycine max) BI315949 (Glycine max) BI203222 (Lycopersicon esculentum) BI176503 (Solanum tuberosum) BG273882 (Grape berries) AY034958 (Arabidopsis thaliana) AF378187(Oryza sativa) BG790580 (Glycine max) BG790079 (Glycine max) BG726150 (Glycine max) BG654021 (Glycine max) BG653916 (Glycine max) BG653624 (Glycine max) BG652711(Glycine max) BG652507 (Glycine max) BG649914 (Glycine max) BG649831 (Glycine max) AJ309093 (Pinus pinaster) BG507656 (Glycine max) BG405632 (Glycine max) BG405474 (Glycine max) BG405204 (Glycine max) BG405017 (Glycine max) BG363730 (Glycine max) BG362638 (Glycine max) BG359764 (Glycine max) BG359452 (Glycine max) BG359307 (Glycine max) AJ311496 (Pisum sativum) BG239317 (Glycine max)

BG237287 (Glycine max) BG157592 (Glycine max) BG155900 (Glycine max) BG047402 (Glycine max) BG046717 (Glycine max) BG046686 (Glycine max) BG046043 (Glycine max) BG043243 (Glycine max) BG042159 (Glycine max) BG041814 (Glycine max) AB045710 (Pyrus pyrifolia) BF597682 (Glycine max) BF597330 (Glycine max) BF597258 (Glycine max) BF595837 (Glycine max) BF595646 (Glycine max) BF425609 (Glycine max) BF423725 (Glycine max) BF324657 (Glycine max) BF154308 (Solanum tuberosum) BF154187 (Solanum tuberosum) BF154037 (Solanum tuberosum) BF153500 (Solanum tuberosum) BF153341 (Solanum tuberosum) BF153335 (Solanum tuberosum) BF153307 (Solanum tuberosum) BF097021 (Lycopersicon esculentum) BF070782 (Glycine max) BF070666 (Glycine max) BF070277 (Glycine max) BF070188 (Glycine max) BF068815 (Glycine max) BF071174 (Glycine max) BF067110 (Glycine max) BF009756 (Glycine max) BE806119 (Glycine max) BE805996 (Glycine max) BE805178 (Glycine max) BE800976 (Glycine max) BE800941(Glycine max) BE800227 (Glycine max) BE611805 (Glycine max) BE611493 (Glycine max) BE610447 (Glycine max) BE610325 (Glycine max) BE609887(Glycine max) BE609881 (Glycine max) BE607382 (Oryza sativa) BE607326 (Oryza sativa) BE607323 (Oryza sativa) BE556484 (Glycine max) BE556306 (Glycine max) BE555319 (Glycine max) AJ001117 (Triticum aestivum) AJ292758 (Anabaena variabilis) BE474178 (Glycine max) BE440931 (Glycine max) BE440323 (Glycine max) BE347277 (Glycine max) AF263384 (Saccharum officinarum) BE330345 (Glycine max) BE211050 (Glycine max) BE209707 (Glycine max) AW561929 (Gossypium hirsutum) BE040389 (Oryza sativa) BE040121 (Oryza sativa) BE039563 (Arabidopsis thaliana) BE034387 (Mesembryanthemum crystallinum) BE033387 (Mesembryanthemum crystallinum) BE023957 (Glycine max) BE023630 (Glycine max) BE022760 (Glycine max) BE021167 (Glycine max) BE020760 (Glycine max) BE020591 (Glycine max) BE020550 (Glycine max) AW990923 (Euphorbia esula) AW832022 (Glycine max) AW760552 (Glycine max) AW756812 (Glycine max) AW756072 (Glycine max) AW756065 (Glycine max) AW734901(Glycine max) AW707163 (Glycine max) AW706660 (Glycine max) AW706520 (Glycine max)

AW706203 (Glycine max) AW705595 (Glycine max) AW704619 (Glycine max) AB025778 (Citrus unshiu) AB021745 (Citrus unshiu) AW666333 (Glycine max) AW666277 (Glycine max) AW666250 (Glycine max) AW620859 (Glycine max) AW598476 (Glycine max) AW598473 (Glycine max) AW597690 (Glycine max) AW597373 (Glycine max) AW597332 (Glycine max) AW596993 (Glycine max) AW570615 (Glycine max) AW570577 (Glycine max) AW570566 (Glycine max) AW570513 (Glycine max) AW569822 (Glycine max) AW568526 (Glycine max) AW568333 (Glycine max) AW509231 (Glycine max) AW472408 (Glycine max) AW459606 (Glycine max) AW458318 (Glycine max) X82504 (C.rubrum) AW432731(Glycine max) AW432392 (Glycine max) AW432192 (Glycine max) AW397142 (Glycine max) AW397071 (Glycine max) AW309503 (Glycine max) AW307502 (Glycine max) AW307391 (Glycine max)AW307001(Glycine max) AW306834 (Glycine max) AB018561 (Citrullus lanatus) AB029401 (Citrus unshiu) AB022092 (Citrus unshiu) AB022091 (Citrus unshiu) AW279073 (Glycine max) AW279053 (Glycine max) AW278487 (Glycine max) AJ388994 (Medicago truncatula) AJ388888 (Medicago truncatula) AW234887 (Glycine max) AJ238219 (Triticum aestivum) AJ238218 (Triticum aestivum) AJ238217 (Triticum speltoides) AW201670 (Glycine max) AW185801(Glycine max) AW185627 (Glycine max) AJ249624 (Triticum aestivum) AJ249623 (Triticum aestivum) AW164630 (Glycine max) AW164393 (Glycine max) AW133248 (Glycine max) AW101578 (Glycine max) AW100191 (Glycine max) AW100069 (Glycine max) AW099557 (Glycine max) X96938 (T.gesneriana) X96939 (T.gesneriana) AW035186 (Lycopersicon esculentum) AW033439 (Lycopersicon esculentum) AW032339 (Lycopersicon esculentum) AJ132002 (Craterostigma plantagineum) AJ132001 (Craterostigma plantagineum) AJ132000 (Craterostigma plantagineum) AJ131999 (Craterostigma plantagineum) AI973811 (Glycine max) AI973710 (Glycine max) AI973540 (Glycine max) AI967739 (Lotus japonicus) AI965972 (Glycine max) AI960742 (Glycine max) AI960703 (Glycine max) AI930917 (Glycine max) AI900130 (Glycine max) AI900087 (Glycine max) AI855470 (Glycine max) AA080634 (Saccharum sp.) AA080610 (Saccharum sp.) AA269294 (Saccharum sp.)

AA080580 (Saccharum sp.) AI736370 (Glycine max) AI731292 (Gossypium hirsutum) AI731115 (Gossypium hirsutum) AI729201 (Gossypium hirsutum) AI728436 (Gossypium hirsutum) AI727966 (Gossypium hirsutum) AI726092 (Gossypium hirsutum) U73588 (Gossypium hirsutum) U73587 (Gossypium hirsutum) AJ012080 (Pisum sativum) AJ131964 (Medicago truncatula) AJ131943 (Medicago truncatula) AJ133726 (Lotus japonicus) Y16091 (Daucus carota) Y16090 (Daucus carota) AJ011319 (Lycopersicum esculentum) AI496671 (Glycine max) AI496540 (Glycine max) AI496532 (Glycine max) AI495774 (Glycine max) AI495135 (Glycine max) AI495023 (Glycine max) AI494833 (Glycine max) AJ011534 (Lycopersicon esculentum) Y15802 (Hordeum vulgare) AI461126(Glycine max) AI460757 (Glycine max) AI460629 (Glycine max) AI444096 (Glycine max) AI444083 (Glycine max) AI444054 (Glycine max) AI443620 (Glycine max) AI443476 (Glycine max) AI443231 (Glycine max) AI442789 (Glycine max) AI442411 (Glycine max) AI441989 (Glycine max) AI441004 (Glycine max) AI437923 (Glycine max) AI437907 (Glycine max) AI437840 (Glycine max) AJ010639 (Anabaena sp.) AJ011535 (Lycopersicon esculentum) D10266 (Vigna radiata) L03366 (Oryza sativa) AF030231 (Glycine max) M97551 (Vicia faba) AJ000153 (Triticum aestivum) AF079523 (Musa acuminata) AF079851 (Pisum sativum) AJ001071 (Pisum sativum) AF049487 (Medicago sativa) AF054446 (Mesembryanthemum crystallinum) AA753339 (Oryza sativa) AA752298 (Oryza sativa) AA752293 (Oryza sativa) AA753445 (Oryza sativa) AA753437 (Oryza sativa) AA753297 (Oryza sativa) AA752123 (Oryza sativa) AA751990 (Oryza sativa) AA750692 (Oryza sativa) AA750079 (Oryza sativa)AA749692 (Oryza sativa) AA749554 (Oryza sativa) AA720478 (Mesembryanthemum crystallinum) AA661050 (Medicago truncatula) AA661041 (Medicago truncatula) AA660686 (Medicago truncatula) D88412 (Cotton) D10418 (Rice) D21308 (Rice) D29733 (Rice) X81974 (B.vulgaris) X92378 (A.glutinosa) Z56278 (V.faba) Z48640 (V.faba) X98598 (P.sativum) T25261 (Zea mays) T23326 (Zea mays) T14713 (Zea mays) T14662 (Zea mays) T14661 (Zea mays) X75332 (D.carota) X02382 (Zea mays) X02400 (Zea mays) X70990 (A.thaliana) X60987 (A.thaliana)

X69773 (V.faba) X73477 (S.tuberosum) Z11532 (S.officinarum) Z15028 (O.sativa) X64770 (O.sativa) X59046 (O.sativa) X66728 (H.vulgare) X65871 (H.vulgare) X69931(H.vulgare) A27685 (O.sativa) W21612 (Zea mays) U24088 (Solanum tuberosum) U24087 (Solanum tuberosum) X73221 (H.vulgare) L32898 (Zea mays) F13913 (Arabidopsis thaliana) F13912 (Arabidopsis thaliana) U21129 (Solanum tuberosum) M26672 (Triticum aestivum) M26671 (Triticum aestivum) L19762 (Lycopersicon esculentum) M18745 (Potato) L33244 (Zea mays) L22296 (Zea mays) Z17959 (Arabidopsis thaliana). These sequences are incorporated by reference.

In a second aspect, the present invention provides a method of altering the production of fibre, fruit or seeds in a plant, the method comprising causing under- or overexpression of an iso-form of SuSy involved in fruit/seed production in the plant.

Preferably, for underexpression, the method involves providing a genetic construct which targets the 3' end of the SuSy gene when transformed in a plant. By "3'-end" is meant a sequence encoding the C-terminal portion of a SuSy polypeptide with or without the 3' non-coding sequence, or a sequence that is complementary thereto. The construct can be a co-suppression antisense or combined sense/antisense (inverted repeat) SuSy construct.

In a third aspect, the present invention provides a plant having an altered ability to produce fibre, fruit or seeds produced by the method according to the second aspect of the present invention.

In a fourth aspect, the present invention provides a genetic construct targeting the 3' end of the SuSy gene which, when expressed in a plant, reduces the expression of an iso-form of SuSy involved in fruit/seed production.

Preferred embodiments of the chimeric genes, particularly of the coding regions used for these methods are as described elsewhere in this specification.

Preferably, the construct is selected from the constructs shown in Figure 5, a combination of parts of both constructs to generate an inverted repeat gene suppression construct, with or without a tissue specific promoter region.

One preferred method of developing constructs is by using the "pPLEX" technology involving subclover stunt virus promoters and terminators described in AU 689311.

In a fifth aspect, the present invention provides use for of the construct according to the fourth aspect of the present invention to produce a plant having reduced ability to produce fibre, fruit or seeds.

The present invention is suitable for the following applications:-

A) Use of SuSy to enhance or otherwise improve fibre yield

-SuSy may be used to enhance or otherwise improve fibre synthesis by increasing SuSy expression or activity in such plant cells e.g. a fibre specific or fibre enhanced promoter (such as but not limited to a cotton expansion promoter, or the promoter of the SuSy gene having the nucleotide sequence of SEQ ID NO: 1) or a constitutive promoter (such as CaMV 35S) can be used to express the SuSy gene in cotton cells.

-Cotton fibre yield can be improved by reducing or suppressing cotton fuzz fibre. Fuzz fibre specific or enhanced promoter or constitutive promoter to reduce sucrose synthase selectively in cotton fuzz fibre cells. Reduced expression can be achieved by gene silencing technologies (antisense, co-suppression, dominant negative etc). Dominant negative mutant alleles of sucrose synthase genes may be obtained e.g. through mutation (insertion, substitution, or deletion of the phosphorylation site(s) of the sucrose synthase protein coding region)

- Cotton fibre yield can be improved by modifying fuzz fibres into lint fibres e.g. by expression of SuSy coding region or a functional equivalent thereof by fibre specific or fibre enhanced promoter, primary or secondary cell wall promoter or a constitutive promoter.

B) Use of SuSy gene to improve fibre quality

-Fibre length is an example of fibre quality. Expression of SuSy by a fibre specific or fibre enhanced promoter, primary cell wall promoter or a constitutive promoter.

C) Use of SuSy to improve fibre properties (e.g. fibre strength, length and number)

-Fibre strength is an example of a fibre property. Fibre strength is significantly effected by the cellulose content in the secondary cell wall of fibre cells. This may be achieved by expression of SuSy by a fibre specific or fibre enhanced promoter, secondary cell wall promoter or a constitutive promoter.

D) Overexpression of SuSy specifically in seeds to increase sucrose utilisation in seeds for increased seed size and storage product content.

E) Overexpression in the maternal tissue of a fruit (such as cotton fibre, horticultural fruit) for increased carbohydrate or fibre content.

F) Suppression of SuSy in seed with over expression of SuSy in fruit/fibre for increased fibre/fruit yield.

Preferred plant-operable promoters include the fibre specific and/or secondary cell wall specific promoters which can be isolated according to the teaching of WO 98/18949, WO98/00549 or US5932713.

The correlation of sucrose synthase activity in the ovule epidermis of cotton with fibre length also allows the use of sucrose synthase proteins (or antibodies or aptamers recognizing the same) or sucrose synthase coding regions as a diagnostic tool in cotton breeding. These may be used to identify cotton lines or varieties, including wild sources, which have enhanced sucrose synthase activity and increased potential to form longer fibres, particularly when crossed in a breeding program with other cotton lines and/or varieties having good fibre characteristics. Nucleic acids derived from sucrose synthase coding regions may be used to determine the amount of sucrose synthase RNA in the ovule epidermis. Also, polymorphism, including single nucleotide polymorphism between sucrose synthase genes may be used to identify lines with superior sucrose synthase alleles.

The methods and means of the invention are particularly suited for use in cotton plants, (both Gossypium hirsutum and Gossypium barbadense) particularly for Coker 312, Coker310, Coker 5Acala SJ-5, GSC25110, FiberMax 819 , Siokra 1-3, T25, GSA75, Acala SJ2, Acala SJ4, Acala SJ5, Acala SJ-C1, Acala B1644, Acala B1654-26, Acala B1654-43, Acala B3991, Acala GC356, Acala GC510, Acala GAM1, Acala C1, Acala Royale, Acala Maxxa, Acala Prema, Acala B638, Acala B1810, Acala B2724, Acala B4894, Acala B5002, non Acala "picker" Siokra, "stripper" variety FC2017, Coker 315, STONEVILLE 506, STONEVILLE 825, DP50, DP61, DP90, DP77, DES119, McN235, HBX87, HBX191, HBX107, FC 3027, CHEMBRED A1, CHEMBRED A2, CHEMBRED A3, CHEMBRED A4, CHEMBRED B1, CHEMBRED B2, CHEMBRED B3, CHEMBRED C1, CHEMBRED C2, CHEMBRED C3, CHEMBRED C4, PAYMASTER 145, HS26, HS46, SICALA, PIMA S6 and ORO BLANCO PIMA.

Any description of prior art documents herein is not an admission that the documents form part of the common general knowledge of the relevant art in Australia.

In order that the present invention may be more clearly understood preferred forms will be described with reference to the following examples and accompanying drawings.

Modes for Carrying Out the Invention

General molecular biology

Unless otherwise indicated, the recombinant DNA techniques utilised in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

Analysis for presence of the transgene by Southern blotting was carried out as described in Sambrook *et al.* (1989) and immunolocalisation of the SuSy protein was carried out on tissue sections as described in Ruan and Chourey (1998).

Gene/DNA isolation

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue or organisms believed to express the gene mRNA and to express it at a detectable level. The gene sequences can also be obtained from a genomic library or genomic DNA.

Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognise and specifically bind the protein; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a hybridising gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or hybridising DNA including expressed sequence tags and the like; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*

An alternative means to isolate a gene encoding is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook *et al.* This method requires the use of oligonucleotide probes that will hybridise to the gene.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimised. The actual nucleotide sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of the gene. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is known. The oligonucleotide must be labelled such that it can be detected upon hybridisation to DNA in the library being screened. The preferred method of labelling is to use ^{32}P-labelled dATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods

may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labelling.

Nucleic acid having all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

Another alternative method for obtaining the gene of interest is to chemically synthesise it using one of the methods described in Fingels *et al.* (Agnew Chem. Int. Ed. Engl. *28:* 716-734, 1989). These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

Cotton Transformation

Cotton was transformed using an Agrobacterium mediated transformation technique as described in F. Murray *et al.* 1999 (Molec. Breeding 5: 219-232). Transformed plants were grown in a glasshouse under natural illumination at day/night temperatures of approximately $30^{0}C$ / $22^{0}C$.

Example 1: Suppression of SuSy expression in cotton.

To provide evidence regarding the role of SuSy in fibre development, the present inventors transformed cotton with co-suppression and antisense SuSy constructs, targeting the 3' end of the seed SuSy cDNA driven by a constitutive subclover stunt seven virus promoter (see Figure 5 for a schematic

representation). The presence of the transgene in 11 transgenic lines so far was confirmed by Southern analysis (see Figure 6). Among them two antisense lines (294-147, 292-43) and one co-suppression line (295-82) showed dramatic reductions in fibre and seed development with the remaining lines showing various degrees of inhibition of fibre growth.

Immunolocalization analysis on 0-d ovule sections (Figure 2) revealed that, compared to wild type fibre initials, SuSy protein was reduced to less than 20% wild type levels in fibres of line 294-147 and to undetectable levels in 295-82. Furthermore, the number and size of initiating fibres was reduced by at least 50% in these two lines as compared that in the wild type ovules. Indeed, by 6 days after anthesis (DAA), these transgenic seeds were virtually fibre-less (see Figure 1). Sucrose synthase activity was determined biochemically. Whereas WT plants show a SuSy activity of 22.5 nmol/min/seed, line 147 showed a SuSy activity of 5.8 nmol/min/seed and line 82 showed no detectable activity. This led to a dramatic reduction of normal fibre initial cells in the ovule epidermis. It is clear from these results that the degree of fibre initiation depends on the level of SuSy expression in the ovule epidermis.

The impact of SuSy expression on fibre initiation is further demonstrated by scanning electron microscopy analysis of the ovule epidermis of line 147 (see figure 7) revealed that many fibre cells are collapsed and shrunk (see figure 7B). Some fibre cells appeared, but these were much smaller in size, and were reduced in number when compared to wild type ovule (figure 7A). The shrunk and collapsed phenotype was clearly visible under high magnification (figure 7C).

These results demonstrate that some iso-forms of SuSy play a critical role in fibre initiation and elongation. Suppression of an iso-form of SuSy can thus be used to prevent or reduce fibre initiation and elongation while overexpression can thus be used to enhance fibre initiation and elongation.

While all the bolls in line 295-82 and 292-43 dropped off prematurely by 10 DAA, some bolls of line 294-147 were retained to maturity. In those mature bolls, most seeds were stunted, shrunken and fibre-less. About 15% of the seeds, however, showed 30% of the wild type fibre length (loose fibre as well) and wild type embryo and seed size, most likely due to segregation of the transgene.

This demonstrates that suppression of iso-forms of SuSy in the maternal tissue (seed coat/ fibre) alone can inhibit fibre development by ~70% in length, while additional repression of SuSy in the embryo can arrest seed development entirely.

Gene suppression of iso-forms of SuSy in the embryo specifically to give a seedless phenotype (in a wide range of horticultural crops such as grape, peach, pear, apple).

Line 292-43 and 295-82 were male-sterile (no pollen) and the seed set can be recovered by pollination with wild-type pollen. This shows SuSy plays a role in male sterility of cotton.

Gene suppression of SuSy in male floral parts was found to cause sterility. Sucrose synthase activity, the fresh weight of pollen and viability of the pollen were tested on developing anthers 2 days before flowering in different lines. The viability assay was conducted by staining with 2,3,5-triphenyltetrazolium chloride (TTC). The results are summarized in Table 1.

Table 1. Suppression of SuSy in anthers leads to male sterility by pollen formation inhibition.

Line	SuSy activity (nmol min^{-1} mg^{-1})	Pollen fresh weight (% of WT pollen per anther)	Viable pollen (% of 100 grains)
WT	44.1 ± 1.1	100.0	85.2
147#1	29.5 ± 6.9	30.0	52
147#2	14.7 ± 0.9	0.0	0.0
82	18.2 ± 1.5	0.0	0.0

A reduction of SuSy activity in anthers by about 33% results in an about 70% reduction of total pollen weight, largely due to the reduction of the number of pollen produced. Of the residual 30%, only half of the pollen was viable. When SuSy was reduced by about 60% no pollen was any longer formed. Pollen development is thus very sensitive to changes in SuSy activity. Pollen formation can thus be influenced (inhibited or promoted) by suppressing or overexpressing SuSy in anthers.

Suppression of SuSy activity in the maternal seed coat tissue in the transgenic cotton plants, also led to reduced fuzz length in the SuSy suppressed transgenic plants when compared to a wild type plant (see Figure 9). Fuzz fibre length was measured from the chalazal end of the mature seeds and the measurement was done by gently stretching the fuzz of seeds, delinted by hand, and measuring the length from the seed coat epidermis to the tip. The brown color visible in the transgenic line, is due to the fact that fuzz is shorter in transgenic lines than in WT, where the brown seed coat is well covered by relatively long fuzz. SuSy activity was also determined. The results can be summarized as follows:

- the wild type plant had a fuzz length of 2.2 mm (and a SuSy activity measurement set at 100%)

- transgenic line 147#2 had a fuzz length of 1.4 mm (and a SuSy activity measurement of 36% of the WT).

From the general correlation detected between SuSy expression and fibre content and/or quality described herein, it will be understood that overexpression of SuSy gene will have potential beneficial effects on fibre, fruit and seed production in plants.

Example 2. Analysis of progeny of cotton lines 82, 147 and 43 with suppressed SuSy activity.

To confirm the correlation between the presence of the SuSy suppression transgene and the reduced fibre initiation in cotton, further analysis was conducted on the T1 generation. About 24 T1 seeds were sown from 6 T0 lines. All germinated seedlings were screened by PCR for presence or absence of the transgenes (nptII; S7 promoter and SuSy suppression transgene). Six PCR positive and one PCR negative segregating individuals from each of the 6 lines were further analysed on molecular biochemical and cellular level. Line 82 was propagated vegetatively as a reference point. The fibre-less phenotype in the different lines of the T0 generation (indicated by arrows in figure 6A) was still preserved in respectively 3 and 2 segregating individuals of the T1 generation for line 147 and 43 respectively (arrows in Figure 6B). The remaining individuals show various degree of fibre/ seed suppression. The vegetatively-kept line 82 continues to produced fibreless seeds. These results demonstrated that the fibreless/ or seed suppressed phenotype described was indeed due to the presence of SuSy transgene.

A positive strong correlation could be found between the degree of sucrose activity in the seed epidermis and the fibre length in segregating T1 generation plants of lines 147, 43 and 101 (see Figures 8, 9 and 10). When fibre length was plotted against sucrose synthase activity for the different plants of the different lines as well as the wild type line, a linear regression could be derived

(Y=2.634X-50; R^2=0.98). The linear nature of the correlation up to the wild-type level, indicates that overexpression of the sucrose synthase activity above wild type level will lead to fibre length greater than fibre length in wild type plants.

A similar strong correlation exists between the reduction in SuSy activity and mature cotton fibre length and dry weight. SuSy activity in seed coat epidermis has been reduced to 28.8%, 42.5% and 53.9% of the wild type level in segregating individuals #1, #2 and #3 of line 43 T1 generation plants respectively. Lint fibre dry weight per boll was 11.1%, 51.6%, and 70.9% respectively.

Cellulose content in cotton fibre is also closely correlated to the level of SuSy expression. Cellulose content was analysed by labelling cotton fibre at day 25 (peak of cellulose synthesis in vivo) with Calcofluor white, a fluorescent dye specifically binding to cellulose. The fluorescent intensity of the labelled fibres, indicating cellulose content, was significantly reduced in the SuSy suppressed transgenic lines when compared to fluorescence in the wild type. The degree of fluorescence reduction correlated to the degree of suppression of SuSy activity.

Example 3. Overexpression of sucrose synthase in cotton plants.

The coding sequence of a potato sucrose synthase cDNA (Genbank Accession number M18745) is operably linked to a subterranean clover stunt virus promoter (S7; WO9606932) and a 3' transcription termination and polyadenylation signal functional in plants. This chimeric gene is operably linked to a selectable marker gene and introduced into a T-DNA vector. Cotton plants are transformed using the above mentioned Agrobacterium mediated transformation technique. Transgenic cotton lines are identified, sucrose synthase activity, fibre length, fuzz fibre length, cellulose content, and dry weight of the lint is analyzed. A positive correlation is found between SuSy activity and increased fibre length cellulose content, and dry weight of the lint.

SUMMARY OF THE EXAMPLES

The single-cell cotton fibres initiate from ovule epidermis at anthesis, elongate to 2.5 ~ 3.0 cm in about 16 days and then synthesize massive amounts of cellulose. Thus, cotton fibre is an excellent system for the study of cell differentiation, elongation and cellulose synthesis in higher plants with significant industry implications for improving fibre yield and quality.

To provide definitive evidence regarding the role of SuSy in fibre and seed development, the present inventors transformed cotton with co-suppression and antisense SuSy constructs, targeting the 3' end of the seed SuSy cDNA driven by subclover stunt seven virus promoter. The presence of the transgene in 9 transgenic lines so far was confirmed by Southern analysis. Among them one antisense line (294-147) and one co-suppression line (295-82) showed dramatic reductions in fibre and seed development with the remaining lines showing various degrees of inhibition of fibre growth. Immunolocalization analysis on 0-d ovule sections revealed that, compared to wild type fibre initials, SuSy protein was reduced to only about 20% wild type levels in fibres of line 294-147 and to undetectable levels in 295-82. Furthermore, the number and size of initiating fibres was reduced by at least 50% in these two lines as compared that in the wild type ovules. Indeed, by 2 d after anthesis (DAA), these transgenic seeds were virtually fibre-less. This is in contrast to a fibre-covered seed phenotype seen in the wild type plants at this stage. While all the bolls in line 295-82 dropped off prematurely by 10 DAA, some bolls of line 294-147 were retained to maturity. In those mature bolls, most seeds were stunted, shrunken and fibre-less. About 15% of the seeds, however, showed 30% of the wild type fibre length and wild type embryo and seed size, most likely due to segregation of the transgene. These results demonstrate (a) that SuSy plays a critical role in fibre initiation and elongation, (b) that suppression of SuSy in the maternal tissue (seed coat/fibre) alone can inhibit fibre development while additional repression of SuSy in the embryo can arrest seed development entirely. The influence of SuSy suppression on the reduction of

fibre length has been confirmed by analysis of progeny of the transgenic cotton lines. Further, a linear correlation was found between the level of SuSy activity and the increase in fibre length. The linear nature of this correlation indicates that in wild type cotton, the level of sucrose synthase is limiting.

Overexpression of sucrose synthase at least in maternal ovule tissue increases fibre length in fibre producing plants.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References:

Amor , Y., Haigler, C.H., Johnson, S., Wainscott, M. and Delmer, D.P. (1995) A membrane associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. *Proc Nat Acad Science USA 92, 9353-9357.*

Ruan, Y.-L., Llewellyn, D.J, Furbank, R.T. (2000) Pathway and control of sucrose import into initiating fibre cells. *Australian Journal of Plant Physiology* 27, 795-800.

Ruan, Y.-L. and Chourey, P.S. (1998) A fibreless seed (fls) mutation in cotton is associated with lack of fibre cell initiation in ovule epidermis, alteration in sucrose synthase expression and carbon partitioning in developing seed. Plant Physiology 118: 399-406

Ruan, Y.-L., Chourey, P.S., Delmer, D.P. and Luis, P.G. (1997) The differential expression of sucrose synthase in relation to diverse patterns of carbon partitioning in developing cotton seed. Plant Physiology 115: 375-385

We claim:

1. A method for altering fibre development or properties of a fibre producing plant comprising the steps of

   a) providing cells of said plants with a chimeric gene comprising the following operably linked DNA fragments:

      (i) a plant operable promoter;

      (ii) a coding region as hereinbefore defined which when transcribed yields an RNA said RNA being capable of reducing the expression of an endogenous sucrose synthase gene or capable of being translated into an active sucrose synthase protein; and

      (iii) transcription termination and polyadenylation signals that function in said plant cells.

2. The method of claim 1 wherein the endogenous sucrose synthase gene is a gene that is expressed in fibre cells.

3. The method of claim 2 wherein the fibre cells are fibre initial cells.

4. The method according to any one of claims 1 to 3, wherein said RNA is capable of being translated into an active sucrose synthase protein.

5. The method according to claim 4, wherein said coding region comprises a nucleotide sequence selected from the group consisting of:

   a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or a fragment of said nucleotide sequence encoding an active sucrose synthase;

b) the nucleotide sequence of SEQ ID NO: 1 or a fragment of said nucleotide sequence encoding an active sucrose synthase;

c) a nucleotide sequence having at least 70% sequence identity to a) or b);

d) a nucleotide sequence that hybridizes under stringent conditions with a) or b) or a complementary sequence thereto or a part thereof encoding an active sucrose synthase.

6. The method according to any one of claims 1 to 3, wherein said coding region is transcribed to produce RNA that is capable of reducing the expression of an endogenous sucrose synthase gene.

7. The method according to claim 6, wherein said coding region comprises a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence comprising at least about 19 contiguous nucleotides of a sequence that has at least 70% sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2;

b) a nucleotide sequence comprising at least about 25 contiguous nucleotides of a sequence having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 1;

c) the complement of a) or b);

8. The method of claim 7 wherein the nucleotide sequence at a) comprises at least about 25 contiguous nucleotides of a sequence that has at least 70% sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

9. The method according to any one of claims 6 to 8, wherein said coding region comprises the nucleotide sequence of SEQ ID NO: 1 from the

nucleotide at about position 2208 to the nucleotide at about position 2598 or the complement thereof.

10. The method according to any one of claims 6 to 9, wherein said coding region comprises both sense and antisense nucleotide sequences capable of forming a double stranded RNA molecule.

11. The method according to any one of claims 1 to 10, wherein said promoter is a subterranean clover stunt virus promoter.

12. The method according to any one of claims 1 to 11, wherein said fibre producing plant is a cotton plant.

13. The method according to claim 12, wherein said cotton plant is a Fibermax™ variety.

14. A method for enhancing fibre yield in a fibre producing plant comprising the steps of:

a) providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments:
   i) a plant operable promoter;
   ii) a DNA region capable of being translated into an active sucrose synthase protein; and
   iii) transcription termination and polyadenylation signals that function in said plant cells.

15. A method for enhancing fibre quality in a fibre producing plant, comprising the steps of

b) providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments

i) a plant operable promoter;

ii) a DNA region capable of being translated into an active sucrose synthase protein

iii) transcription termination and polyadenylation signals that function in said plant cells.

16. A method for increasing seed size in a fibre producing plant, comprising the steps of:

a) providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments

b) a seed-specific promoter;

c) a DNA region capable of being translated into an active sucrose synthase protein; and

d) transcription termination and polyadenylation signals that function in said plant cells.

17. A fibre producing plant comprising in its genome a chimeric DNA comprising the following operably linked DNA fragments:

i) a plant operable promoter;

ii) a coding region which when transcribed yields an RNA said RNA being capable of reducing the expression of an endogenous

sucrose synthase gene or capable of being translated into an active sucrose synthase protein; and

iii) transcription termination and polyadenylation signals that function in said plant cells.

18. The fibre producing plant of claim 17 wherein the coding region is transcribed to yield RNA that is capable of reducing the expression of an endogenous sucrose synthase gene in fibre cells.

19. The fibre producing plant of claim 18 wherein the fibre cells are fibre initial cells.

20. The fibre producing plant according to claim 17, wherein said RNA is translated into an active sucrose synthase protein.

21. The fibre producing plant of claim 20 wherein the active sucrose synthase protein is expressed in fibre cells and wherein said fibre cells have an increased sucrose synthase activity compared to fibre cells of plant cells which do not comprise said chimeric DNA.

22. The fibre producing plant of claim 21 wherein the fibre cells are fibre initial cells.

23. The fibre producing plant according to any one of claims 17 to 22, wherein that coding region comprises a nucleotide sequence selected from the group consisting of:

a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or a fragment of said nucleotide sequence encoding an active sucrose synthase;

b) the nucleotide sequence of SEQ ID NO: 1 or a fragment of said nucleotide sequence encoding an active sucrose synthase;

c) a nucleotide sequence having at least 70% sequence identity to a) or b); and

d) a nucleotide sequence that hybridizes under stringent conditions with a) or b) or a complementary sequence thereto or a part thereof encoding an active sucrose synthase.

24. The fibre producing plant according to claim 23, wherein said coding region comprises a nucleotide sequence selected from the group consisting of

a) a nucleotide sequence comprising at least about 19 contiguous nucleotides of a sequence that has at least 70% sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2;

b) a nucleotide sequence comprising at least about 25 contiguous nucleotides of a sequence having at least 70% sequence identity to the nucleotide sequence of SEQ ID NO: 1;

c) the complement of a) or b);

25. The fibre producing plant of claim 24 wherein the nucleotide sequence at a) comprises at least about 25 contiguous nucleotides of a sequence that

has at least 70% sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

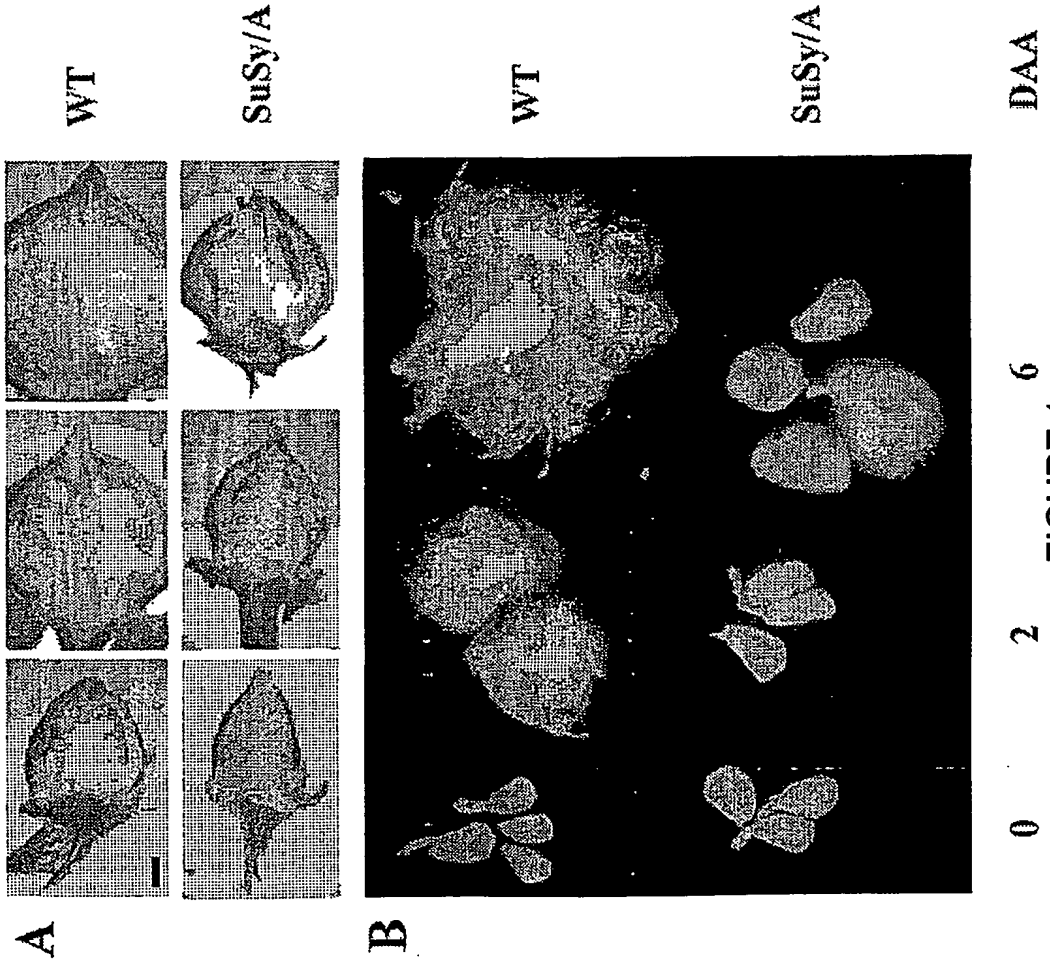
26. The fibre producing plant according to any one of claims 17 to 25, wherein said coding region comprises the nucleotide sequence of SEQ ID No: 1 from the nucleotide at about position 2208 to the nucleotide at about position 2598 or the complement thereof.

27. The fibre producing plant according to any one of claims 17 to 26, wherein said plant is a cotton plant.

28. Seeds of the plant according to any one of claims 17 to 27.

29. Fibre is olated from the plant according to any one of claims 17 to 27 or the seed of claim 28, wherein said fibre has altered development or an altered property relative to the fibre from a plant that does not comprise the chimeric DNA.

30. A plant obtained by performing the method according to any one of claims 1 to 15.



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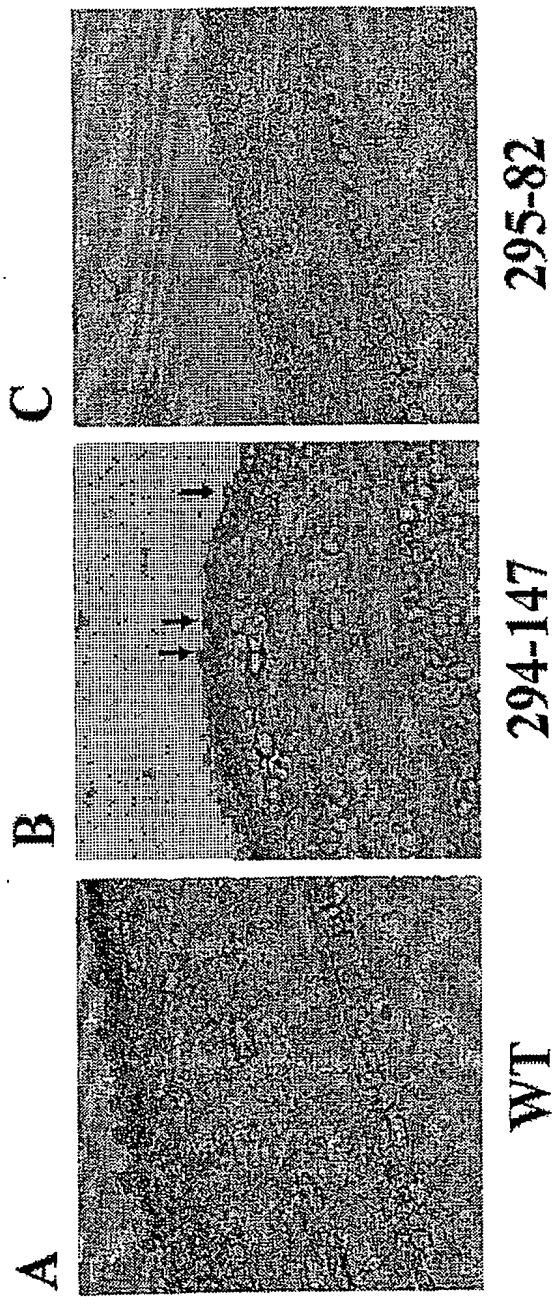

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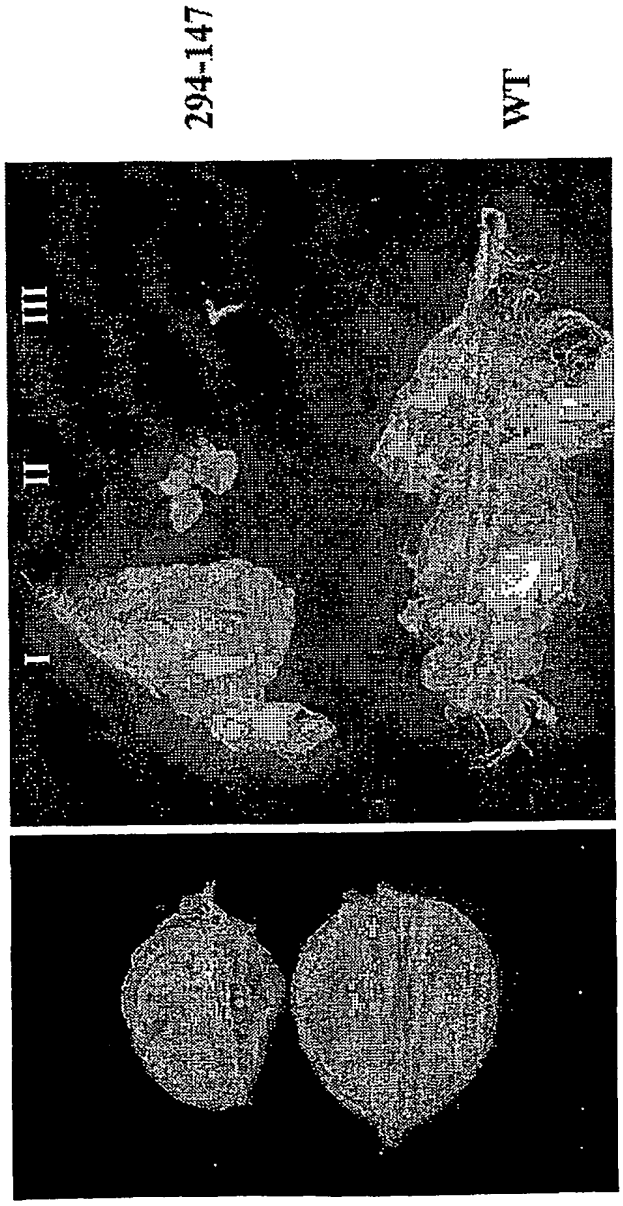
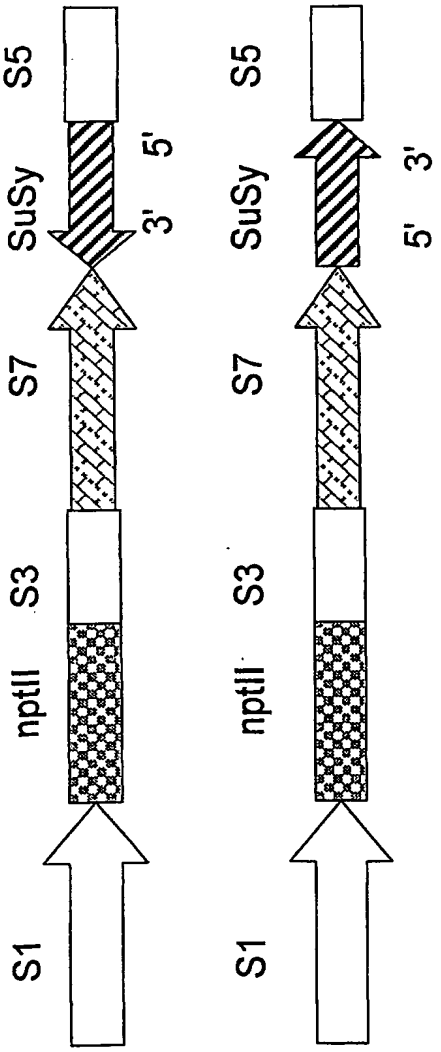


FIGURE 3

FIGURE 4



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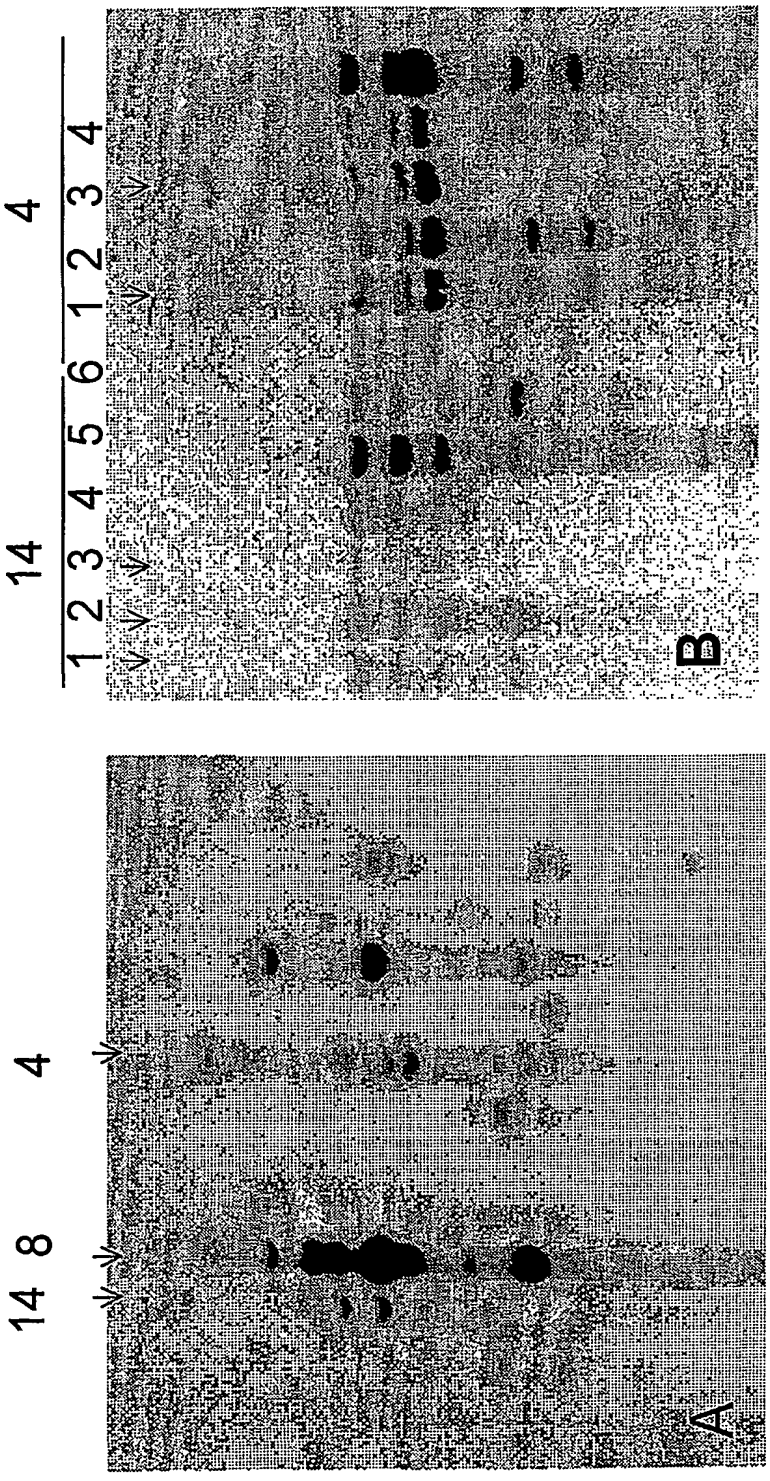

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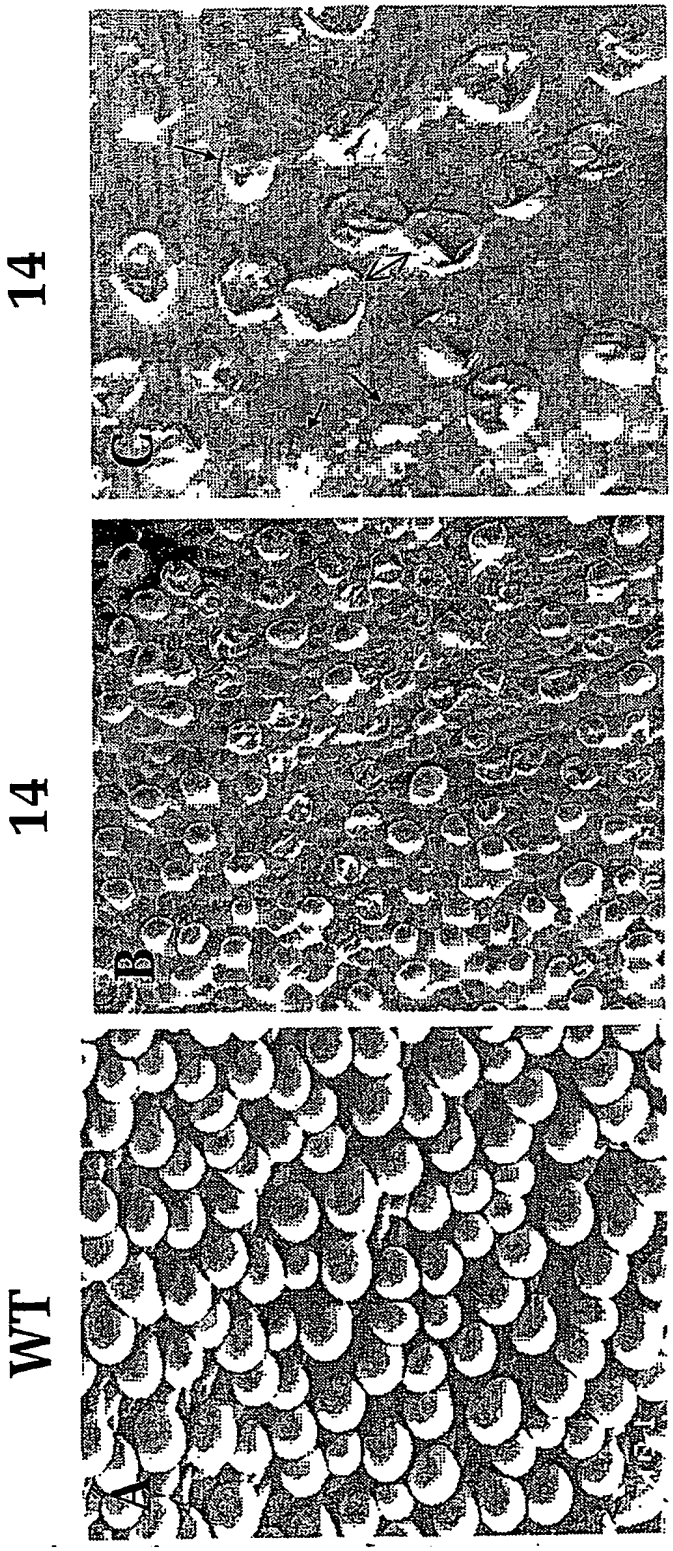

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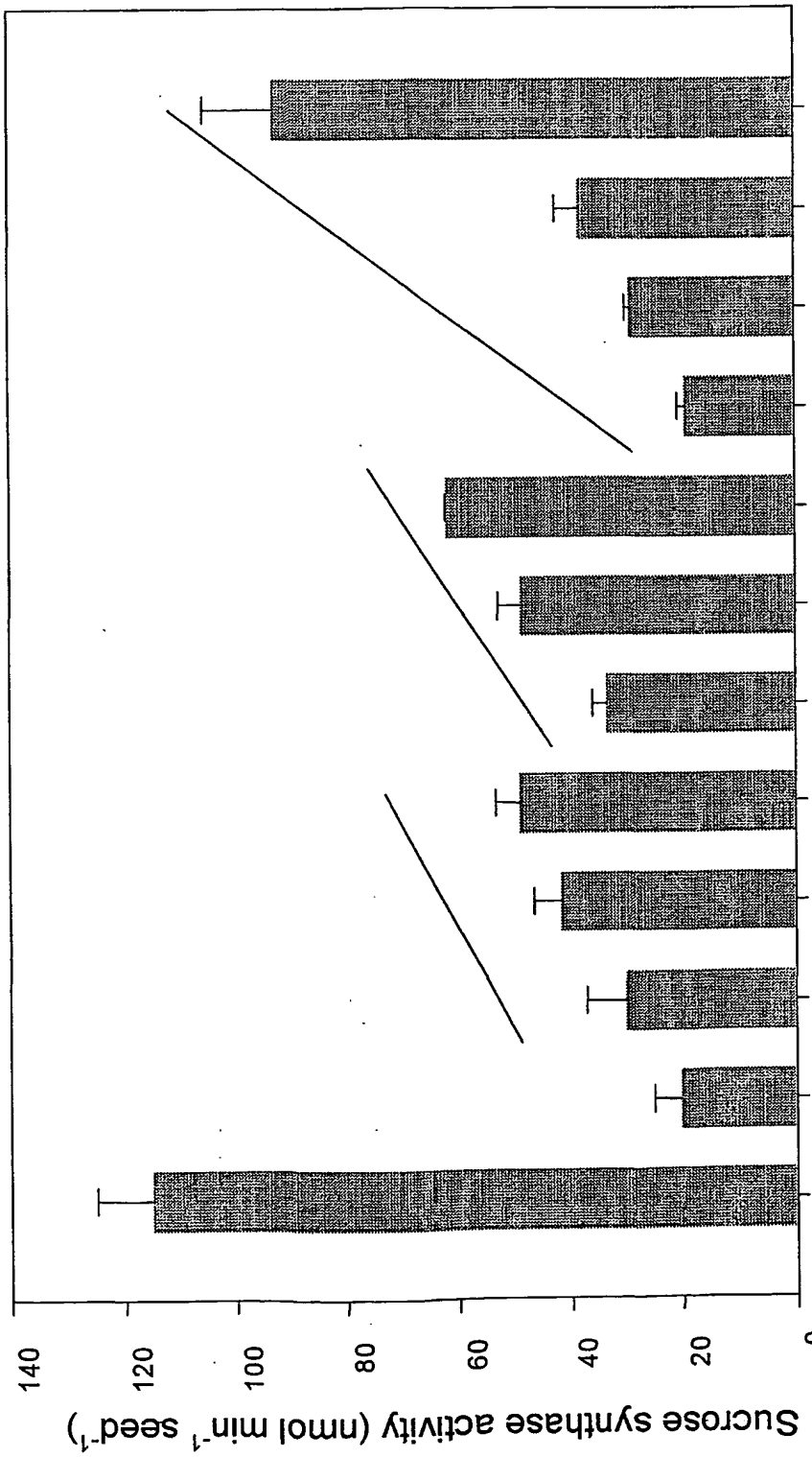
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40
60
80
100
120
140
WT
82-1
147-1
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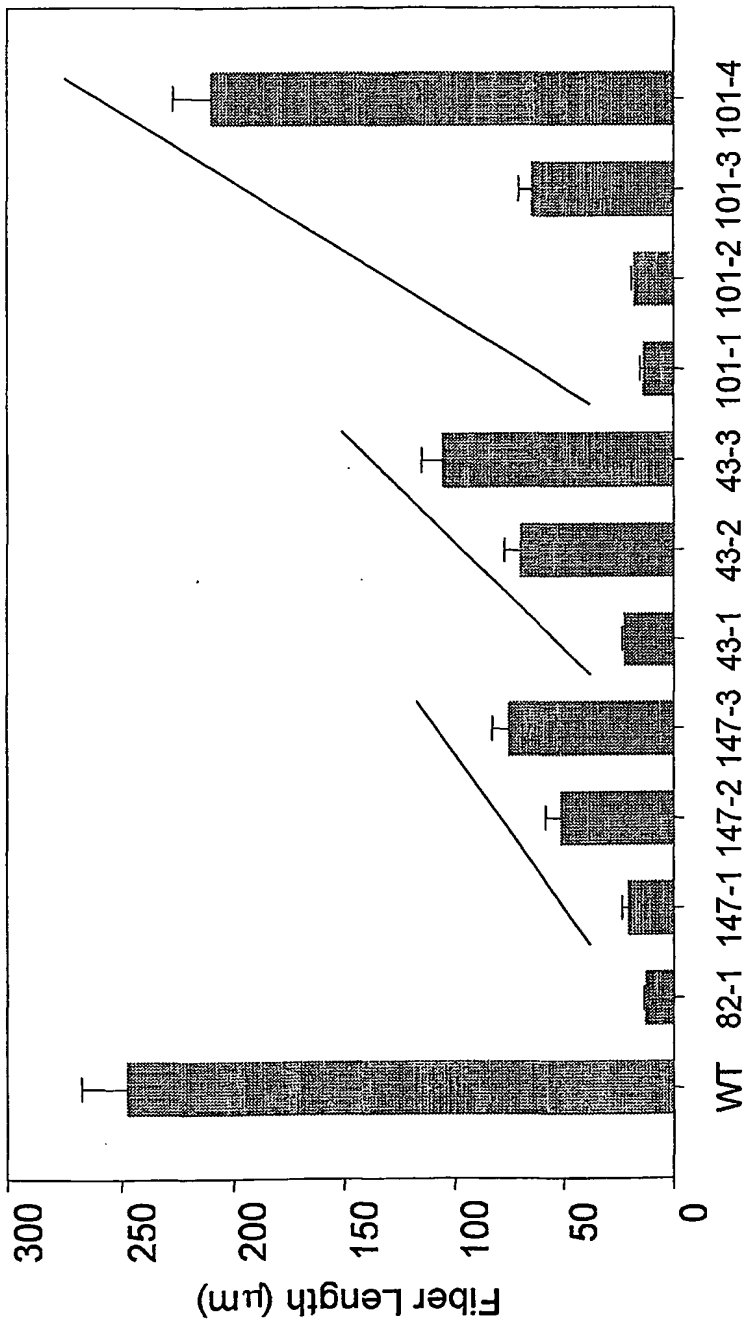
147-3
43-1
43-2
43-3
101-1
101-2
101-3
101-4

FIGURE 8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WT
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147-3
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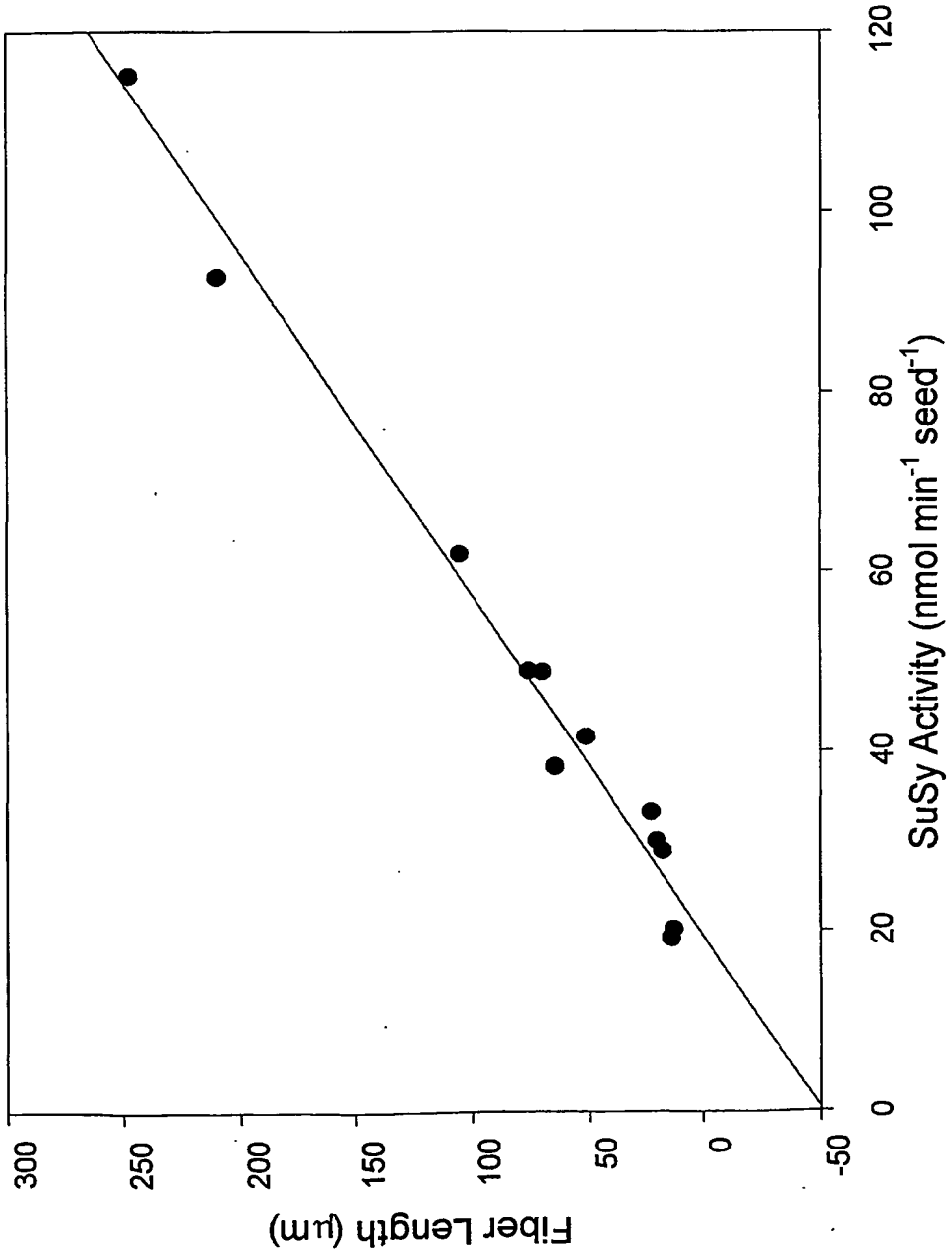
43-2
43-3
101-1
101-2
101-3
101-4

FIGURE 9


Fiber Length (µm)
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250
200
150
100
50
0
-50
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20
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60
80
100
120
SuSy Activity (nmol min-1 seed-1)

FIGURE 10

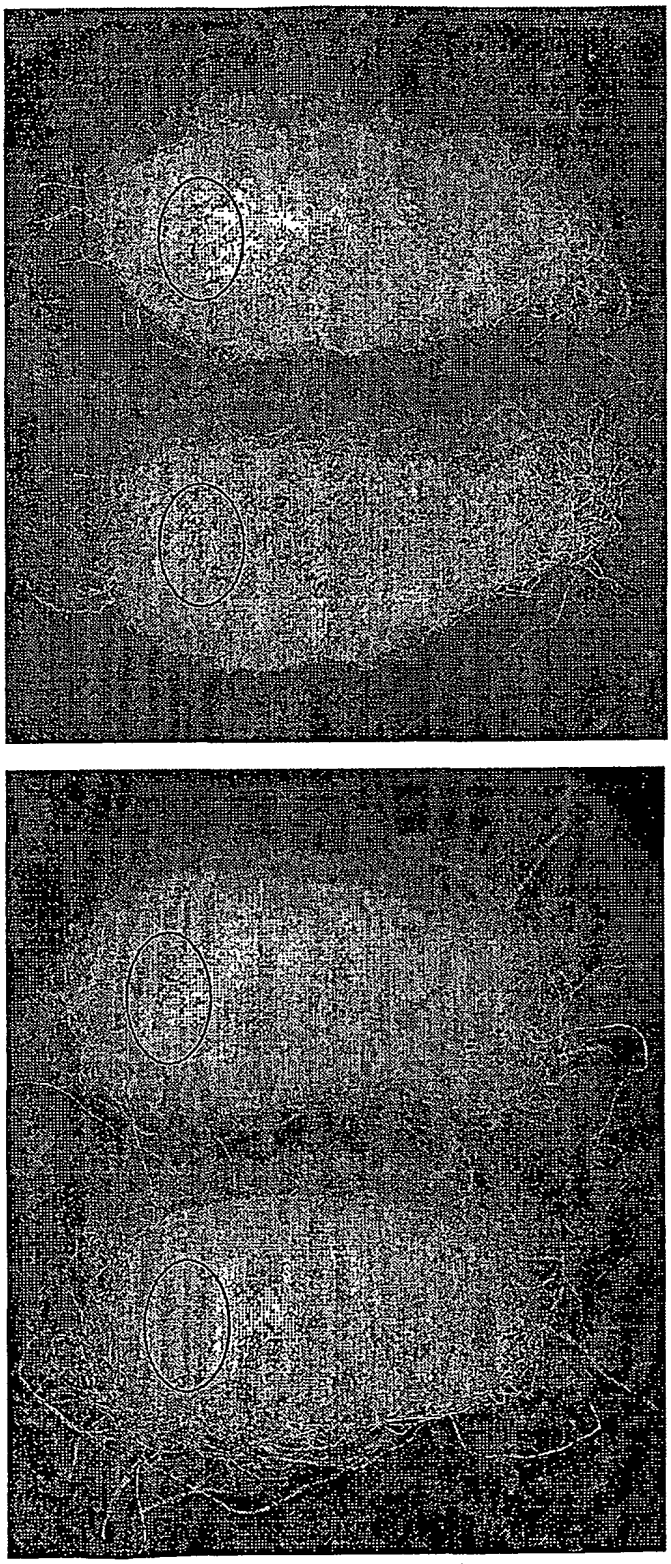

FIGURE 11

SEQUENCE LISTING

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<130> 500159/MRO

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agg atc gag ggc aaa gga aaa gga att ctg caa cac cat caa att att     144
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        35                  40                  45

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Leu Leu Glu Ala Thr Asp Pro Cys Thr Leu Glu Lys Phe Leu Gly Arg
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Ala Gln Asp Asn Val Leu Gly Tyr Pro Asp Thr Gly Gly Gln Val Val
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305                 310                 315                 320

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                325                 330                 335

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Arg Leu Leu Pro Asp Ala Val Gly Thr Thr Cys Gly Gln Arg Leu Glu
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    370                 375                 380

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Trp Pro Tyr Leu Glu Thr Tyr Thr Glu Asp Val Ala His Glu Ile Ser
385                 390                 395                 400

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465                 470                 475                 480

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/01580

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A01H 5/00 C12N 15/11 C12N 15/29 C12N 15/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

WPIDS, CA

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SEE ELECTRONIC DATABASES

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS (A01H + keywords); CA, AGRICOLA, MEDLINE (sucrose synthase, susy, transgen?, transform?, recombinant, plant, cotton, fibre, fiber, seed)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ruan, Y-L. *et al.* 2000. Pathway and control of sucrose import into initiating cotton fiber cells. Australian Journal of Plant Physiology. 27:795-800. See whole document, in particular "Results and discussion" pp797-800.	1-30
X	Ruan, Y-L. and Chourey, P.S. 1998. A fiberless seed mutation in cotton is associated with lack of fiber cell initiation in ovule epidermis and alterations in sucrose synthase expression and carbon partitioning in developing seeds. Plant Physiology. 118:399-406. See whole document, in particular "Results" and "Discussion" pp400-405.	1-13, 17-30
A	Chengappa, S. *et al.* 1999. Transgenic tomato plants with decreased sucrose synthase are unaltered in starch and sugar accumulation in the fruit. Plant Molecular Biology. 40:213-221. See "Abstract" and "Results and Discussion".	1-13, 17-30

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search: 15 January 2002

Date of mailing of the international search report: 31 JAN 2002

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INTERNATIONAL SEARCH REPORT	International application No. PCT/AU01/01580

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	D'Aoust, M-A. *et al.* 1999. Antisense inhibition of tomato fruit sucrose synthase decreases fruit setting and the sucrose unloading capacity of young fruit. The Plant Cell. 11:2407-2418. See "Abstract" and "Discussion".	1-13, 17-30
A	Ruan, Y-L. *et al.* 1997. The differential expression of sucrose synthase in relation to diverse patterns of carbon partitioning in developing cotton seed. Plant Physiology. 115:375-385. See "Introduction" and "Discussion".	1-30
A	Shimizu, Y. *et al.* 1997. Changes in levels of mRNAs for cell wall-related enzymes in growing cotton fiber cells. Plant and Cell Physiology. 38(3):375-378. See whole document.	1-30
T	Haigler, C.H. *et al.* 2001. Carbon partitioning to cellulose synthesis. Plant Molecular Biology. 47:29-51. See whole document.	1-13, 17-30